#2 Cont

degradation products were identified as listed in Table 4.

Please replace the paragraph at page 33, line 8-12 with the following:

Traces from RP-HPLC and mass spectrometry analyses are shown in Figure 9 and 10. Panel B of Figure 9 shows the single peak obtained from electrospray mass spectrometry of the RP-HPLC peak shown in panel A, isolated from TnC by affinity chromatography.

#3 Analysis of the rat cardiac (rc) TnI amino acid sequence identified a single sequence of appropriate mass, rcTnI residues 1 to 193 of SEQ ID NO:11 (see Table 4) (intact rcTnI has 210 amino acid residues, Figure 17B).

REMARKS

With respect to rat cardiac troponin I residues or fragments disclosed at pages 29-30 and 32-33 of the specification, Applicants have amended the specification in accordance with the Examiner's request to refer to SEQ ID NO:11. With respect to the myosin light chain 1 (MLC1) residues or fragment referred to at page 29 of the specification, Applicants have amended the specification to include a reference to the SWISS-PROT Accession Number for the MLC1 sequence which was available to the public prior to the filing date of this application through

http:\\www.expasy.ch\cgi-bin\sprot-search-de.

No new matter has been added by this amendment and entry of

these amendments is therefore respectfully requested.

Respectfully submitted,

Kathleen A. Tyrrell
Registration No. 38,350

Date: **September 10, 2001**

Licata & Tyrrell P.C.
66 E. Main Street
Marlton, New Jersey 08053
(856) 810-1515

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please replace the paragraph at page 29, line 4, through page 30, line 3 with the following:

Western blot analysis was done according to Van Eyk *et al.* 1998 (*Circ. Res.* 82:261-71) or else the primary antibodies were detected with goat anti-mouse IgG conjugated to alkaline phosphatase (Jandel Scientific) and CDP-Star chemiluminescence reagent (NEN-Mandel). The monoclonal antibodies used were anti-TnT clone JLT-12 (Sigma Chemical Co., St Louis, Mo), anti-α-actinin clone EA-53, (Sigma) or anti-α-actinin clone 157 (provided by Spectral Diagnostics, Toronto, Canada), anti-MLC1 (provided by Abbott Laboratories, Chicago, IL) which recognizes amino acid residues 70 to 75 of SWISS-PROT Accession No. P17209 (http://www.expasy.ch/cgi-bin/sprot-search-de), anti-TM (Sigma), anti-sarcomeric actin (Sigma), and anti-glyceraldehyde phosphate dehydrogenase (Cedarline Lab. Ltd, Canada). Several different anti-TnI antibodies were utilized: anti-TnI clone 3309 which recognizes amino acid residues 157 to 192 of SEQ ID NO:11 and clone AM-NI which recognizes TnI residues 1 to 65 of SEQ ID NO:11 (provided by Dr. J. Ladenson, Washington University St Louis, Mo.), anti-TnI clone 10F2 (MAb 10F2) which recognizes amino acid residues 189 to 199 of SEQ ID NO:11 (see epitope map Figure 8 in Van Eyk *et al.* 1998, *Circ. Res.* 82:261-71), antibody provided by Dr. C. Larue at Univ. Innsbruck Med. School, Austria, MAb C5 (Research Diagnostics, Flanders, NS), and our anti-TnI peptide (P143T) residues 137 to 148 of SEQ ID NO:11 (MAb E2). The production of the anti-TnI peptide monoclonal antibodies

including MAbE2 has been described in Van Eyk *et al.* 1995 (*Prot. Sci.* 4:781-90). MAb E2 recognizes intact skeletal and cardiac TnI and cardiac TnI peptides containing amino acid residues 136 to 148 of SEQ ID NO:11 (data not shown). As well, anti-TnI antibodies MAb 8I-7 and 3I-35 (both Spectral Diagnostics, Toronto, Canada), and MAb C5 (Research Diagnostics, Flander, NS), which recognize TnI amino acid residues (136 to 147, 188 to 199, and 188 to 199 of SEQ ID NO:11, respectively, see McDonough *et al.* 1998, *Biophysical J.* 74:A354). Epitope mapping of these various antibodies was carried out by 12% SDS PAGE of intact cardiac TnI and various TnI fragments followed by western blot analysis as outlined above. Bovine cardiac TnI and rabbit skeletal TnI were purified by HPLC (Ingraham *et al.* 1988, *Biochemistry* 27:5891-98); recombinant rat cardiac TnI fragments 54 to 210, 1 to 188, and 1 to 199 of SEQ ID NO:11 were provided by Dr. A Martin (Univ. Illinois at Chicago, IL; Rarick *et al.* 1997, *J. Biol. Chem.* 272:26887-92), and the synthetic TnI peptide 96 to 142, which is equivalent to the cardiac peptide residues 129 to 175 of SEQ ID NO:11, was prepared by solid-phase peptide synthesis (Tripet *et al.* 1997 *J. Mol. Biol.* 271:728-50).

Please replace the paragraph at page 31, line 23, through page 32, line 4 with the following:

To identify the site of modification in troponin I, specific antibodies to the amino- and carboxyl-termini of troponin I were used to find out which antibodies bind to the different modification products. The antibodies MAb 10F2 (recognizes

residues 188 to 199 of SEQ ID NO:11) and MAb 3350 (2F6.6)(recognizes residue 28 to 54 of SEQ ID NO:11) were used (Van Eyk *et al.* 1998, *Circ. Res.* 82:261-71). The various modification products were run on either a 12% SDS-PAGE or 10% T-PAGE (described in Schagger *et al.* 1987, *Analytical Biochemistry*, 166:368-79). The proteins were transferred to nitrocellulose using a 10 mM CAPS buffer pH 11.0 for 16 h at 27 V (described in Towbin *et al.* 1979, *PNAS* 76:4350-54). The carboxyl-terminus is usually the first to be clipped (Figure 7), yielding residues 1 to 93 of SEQ ID NO:11 (Figure 9), but in addition there are further modifications occurring at the amino-terminus with more severe ischemia (Figure 9). Further TnI degradation products were identified as listed in Table 4.

Please replace the paragraph at page 33, line 8-12 with the following:

Traces from RP-HPLC and mass spectrometry analyses are shown in Figure 9 and 10. Panel B of Figure 9 shows the single peak obtained from electrospray mass spectrometry of the RP-HPLC peak shown in panel A, isolated from TnC by affinity chromatography. Analysis of the rat cardiac (rc) TnI amino acid sequence identified a single sequence of appropriate mass, rcTnI residues 1 to 193 of SEQ ID NO:11 (see Table 4) (intact rcTnI has 210 amino acid residues, Figure 17B).